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Tinospora cordifolia stem extract as an antioxidant additive for enhanced stability of Karanja biodiesel

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ABSTRACT

Oxidative stability of biodiesel is an important fuel quality parameter that not only affects the composition of the fuel but also affects the performance of the engine and tailpipe emissions. The fatty acid profiling of Karanja oil used in this study revealed the presence of ≈69% unsaturated components and the Karanja biodiesel (2.49 h) failed to meet the ASTM D6751 (3 h) and EN 14214 (6 h) specification for oxidation induction period. The utility of *T. cordifolia* stem extract as an antioxidant additive for Karanja biodiesel has been investigated in this work. The extraction experiments were optimised in terms of solvent composition, extraction time and extraction temperature using response surface based Box-Behnken designing approach. Characterisation of the stem extract revealed high total phenolic content with excellent radical scavenging activity. The extract was reasonably soluble in biodiesel, and it was able to extend the oxidation induction period of biodiesel. The ASTM D6751 and EN 14214 specifications were met at a loading of 100 and 600 ppm respectively. The findings of the study indicate that the *T. cordifolia* stem extract can serve as a cheap, environment-friendly and non-toxic alternative of synthetic antioxidants.

1. Introduction

Biodiesel has attracted tremendous research and policy attention as an alternative to fossil fuels, particularly for meeting the demands of the transport sector (Demirbas, 2007). Chemically a mixture of fatty acid alkyl esters (FAAE), biodiesel is usually derived *via* the catalytic transesterification (also known as alcoholysis) of vegetable oil, single cell oil or animal fat and its fuel properties are similar to that of mineral diesel (Demirbas, 2007; Ma and Hanna, 1999). However, large-scale acceptability and marketability of biodiesel are hampered due to its poor stability. The fatty acid composition of biodiesel has a direct effect on its several fuel properties. Unsaturated fatty acids typically constitute a significant fraction of vegetable oil, and it has a favourable effect on the viscosity and cold flow properties of biodiesel but negatively affects its stability and cetane number (Knothe, 2005). Points of unsaturation in fatty acids are susceptible to oxidation upon prolonged storage. Prolonged exposure to air, sunlight, moisture, elevated temperature and presence of metals and other extraneous materials are known to catalytically promote the oxidative degradation of biodiesel (Knothe, 2007).

Oxidative degradation of biodiesel not only alters the composition of the fuel but also affects its fuel properties, engine operation, and performance along with tailpipe exhaust composition (Ashok et al., 2017; Ryu, 2010).

Oxidation of biodiesel involves three sets of mechanisms including initiation of oxidation by the formation of free radicals (Eq. (1)), propagation of free radical chain reaction (Eqs. (2) and (3)) and termination of the chain reaction (Eqs. (4) and (5)) to produce oxidation products (Mittelbach and Schober, 2003).

The European standard for biodiesel (EN 14214) and the American standard (ASTM D6751) specify a minimum oxidation induction period of 6 h and 3 h respectively. The oxidation induction period indicates the time until the oxidation products are first detected during an accelerated oxidation test in which the biodiesel is heated at elevated temperature with constant pumping of air (Lacoste and Lagardere, 2003).

$$RH \rightarrow R^{\cdot} + H^{\cdot} \quad (1)$$

$$R^{\cdot} + O_2 \rightarrow ROO^{\cdot} \quad (2)$$

$$ROO^{\cdot} + RH \rightarrow ROOH^{\cdot} + R^{\cdot} \quad (3)$$

$$R^{\cdot} + R^{\cdot} \rightarrow R{-}R \quad (4)$$

$$R^{\cdot} + ROO^{\cdot} \rightarrow ROOR \quad (5)$$

$$R^{\cdot} + AH \rightarrow R + AH^{\cdot} \quad (6)$$

Oxidative degradation of biodiesel and other oxidisable materials

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can be prevented or delayed by the addition of substances capable of scavenging free radicals, commonly known as antioxidants (Tang et al., 2008). Antioxidants are highly active at very low dosage and function by readily donating an electron or a hydrogen atom to free radicals/reactive oxygen species and thereby protect the target material from getting oxidised (Eq. (6)) (Blokhina et al., 2003). Several types of synthetic compounds such as Propyl Gallate (PG), Pyrogallol (PY), Butylated Hydroxytoluene (BHT), Tertiary-Butylhydroquinone (TBHQ), Octyl Gallate (OG) and Butylated Hydroxyanisole (BHA), have been used as antioxidant additive for biodiesel and they are handy at low dosage in scavenging oxidative species and thereby protect the fuel from deteriorating (Varatharajan et al., 2011). Unfortunately, most of these antioxidants are expensive (Roveda and Trindade, 2018), and have shown carcinogenic and toxic effects (Kahl, 1984). These issues have diverted the attention towards the exploration and development of cheap, environment-friendly and non-toxic alternatives. There has been a tremendous surge in the exploration of plants and parts thereof as a potential storehouse of compounds having antioxidant properties (Ahmad et al., 2010; Pandhair and Sekhon, 2006).

Plant phenolics are the most abundant secondary metabolite involved in several types of plant defence mechanisms such as those against oxidants, parasites, predators, pathogens and ultraviolet radiation (Pereira et al., 2009). Phenolics also known as 'polyphenols' contain an aromatic ring to which one or more hydroxyl groups remain attached. Phenolics can act as antioxidants in several ways; such as by donating hydrogen atoms to reactive oxygen/nitrogen species, by chelating metal cations involved in the free radical generation and by inhibiting some enzymes involved in the production of free radicals (Pereira et al., 2009). Phenolics have been reported to show excellent antioxidant activity *in vitro* (Dai and Mumper, 2010).

Tinospora cordifolia (Family: Menispermaceae) is a herbaceous vine widely distributed in India (Indigenous), Myanmar and China and is considered as one of the most divine plants in Ayurvedic, Folk and Siddha systems of medicine (Panchabhai et al., 2008; Sinha et al., 2004). *T. cordifolia* extracts are known to have antioxidant, anti-inflammatory, chemopreventive, radioprotective, neuroprotective, hypolipidaemic, anti-allergic rhinitis, hepatoprotective, anti-ulcer, cardioprotective, hypoglycemic, and immune-modulatory effects (Mishra et al., 2013; Panchabhai et al., 2008). High-Performance Liquid Chromatography (HPLC) analysis of *T. cordifolia* methanolic stem extract revealed the presence of several phenolic acids such as Caffeic acid, Cinnamic acid, Ferulic acid, and Tannic acid and the extracts showed excellent radical scavenging activity (Singh et al., 2010). The antiproliferative and free radical scavenging activity of *T. codifolia* was impressive, and the plant deserves further investigation (Polu et al., 2017).

Utilization of edible grade vegetable oils as feedstock for biodiesel gives rise to the "*food vs fuel dilemma",* and hence there has been a surge in the exploration of non-edible grade oil for the production of biodiesel (Rathmann et al., 2010). *Millettia pinnata* (commonly known as Karanja) has been identified as one of the most promising oilseed plant (seeds contain 30–35% oil w/w) for biodiesel production as it can grow in relatively arid and infertile substrate and being a legume, it improves the fertility of the land by means of symbiotic N_2 fixation (Kumar and Sharma, 2011; Scott et al., 2008).

Till date, there is no report on the assessment of *T. cordifolia* extracts as an antioxidant additive for biodiesel. Accordingly, in this study, the total phenolic content (TPC) and radical scavenging activity of the *T. cordifolia* stem extracts have been analysed. The extraction efficiency of phenolic compounds depends on several factors including the extraction solvent used, extraction time and extraction temperature. Hence, these variables need to be optimised during extraction experiments to determine their optimal combination at which the extraction yield is highest and also to achieve resource use efficiency. Unlike the best-guess-approach, one-factor-at-a-time approach and other non-statistical approaches for optimising a response variable the Response Surface Methodology (RSM) is a statistically designed optimisation tool which ensures efficient use of the collected data (Montgomery, 2017). Accordingly, RSM based Box-Behnken designing approach was used to optimise the effect of the independent variables on the response (TPC in the extract). The extracts were later added to Karanja biodiesel in different proportions to ascertain its effect on oxidation induction period.

2. Materials and method

2.1. Materials

Emplura grade methanol, sodium carbonate, sodium sulphate, butylated hydroxytoluene (BHT) and sulphuric acid were purchased from Merck India, while analytical grade 2,2-Diphenyl-1-picrylhydrazyl (DPPH·), Folin Ciocalteu's phenol reagent (FC Reagent), and sodium methoxide were purchased from Sigma Aldrich, Merck India and Loba Chemicals Mumbai respectively. Fresh *T. cordifolia* stem was collected locally, and Karanja oil was purchased from a local soap industry situated at Ranchi.

2.2. Extraction optimisation experiments

Fresh *T. cordifolia* stem was chopped into small pieces, and the content of moisture was estimated by heating a weighted portion of the biomass at 105 °C until constant weight. The rest of the biomass was shade dried until the moisture content was reduced to < 2% of the initial moisture content. The dried biomass was ground to fine powder form using agate mortar and pestle, and it was sieved using sieve having a mesh size of 300 μm and the larger fractions were discarded.

Extraction of phenolics from the dried biomass was performed in a capped conical flask using methanol with varying amount of de-ionised water as the extraction solvent. Extraction experiments were also optimised in terms of temperature and time. The solvent to the solute ratio of 5:1 (v/w) and stirring rate of 800 rpm were maintained for all the extraction experiments. The conical flask was fitted with a reflux condenser for all the experiments performed at a temperature > 20 °C. After the stipulated period the contents of the conical flask were subjected to sonication on ultrasonic cleaning bath (Cole-Parmer LK-08895-06) at 40 kHz for 20 min for enhanced recovery of plant phenolics. After ultrasound-assisted extraction, filtration was performed to recover the solid biomass.

The designing and analysis of extraction experiments were performed by Design Expert (Stat-Ease Version 11). Response surface methodology based Box-Behnken designing approach consisting of three factors, three levels, twelve factorial points and five centre points was used for the optimisation of extraction experiments. All analyses were performed within a month of specimen collection.

The content of water in the extraction solvent (methanol), incubation time and incubation temperature were selected as the independent variables while the TPC in the extract was the selected response (dependent variable). The experiments were performed in a randomised order to minimise the role of extraneous factors on unexplained variability. The obtained response was fitted to a second order polynomial quadratic model (Eq. (7)).

$$Y = \beta_o + \sum_{i=1}^{3} \beta_i x_i + \sum_{i=1}^{3} \beta_{ii} x_i^2 + \sum_{i=1}^{2} \sum_{j=i+1}^{3} \beta_{ij} x_{ij} + \varepsilon \quad (7)$$

Where, Y is the response (TPC), βo is the regression coefficient for the intercept, β_i is the regression coefficient for the linear term, β_{ii} is the regression coefficient for the quadratic term and β_{ij} is the regression coefficient for the interactive term, x_i and x_j are independent variables and ε is the error.

The response surface plots were obtained by keeping a variable constant in the quadratic model while the remaining variables were varied. Analysis of variance (ANOVA) with a confidence interval of 95%